PATENT SPECIFICATION

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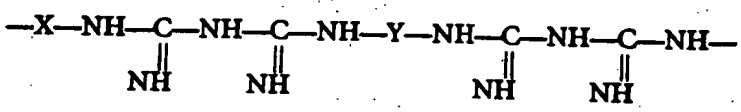
(19)

(54) BIOCIDES

(71) We, IMPERIAL CHEMICAL INDUSTRIES LIMITED, Imperial Chemical House, Millbank, London SW1P 3JF, a British Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to biocides and more particularly to certain biocidal polymeric biguanides and the use thereof.

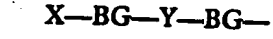
U.K. Specification No. 702 268 discloses polymeric biguanides having a recurring polymeric unit represented by the formula:—



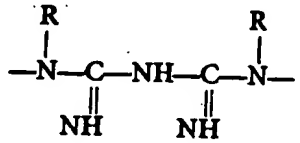
wherein X and Y, which may be the same or different, represent bridging groups $-(CH_2)_n-$ and $-(CH_2)_m-$ respectively, n and m having values from 3 to 12, or X and Y represent other bridging groups in which, taken together, the total number of carbon atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is from 10 to 16, and salts of these compounds. These polymeric biguanides have antibacterial and antifungal properties.

It has now been found that certain other polymeric biguanides are also effective biocides.

According to the present invention there is provided a process for the preparation of polymeric biguanides and salts thereof in which the recurring polymer unit has the general formula:—



wherein BG represents the biguanide radical



in which

each R independently represents a hydrogen atom or a hydrocarbon radical;

X represents a polymethylene radical having from 2 to 12 carbon atoms or a radical the same as Y;

Y represents a divalent radical selected from

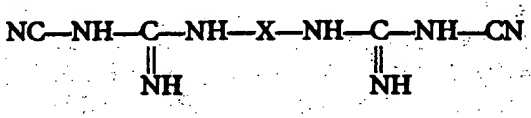
(i) symmetrical aliphatic, aromatic or aliphatic-aromatic radicals having one or two amide or urea linkages therein;

(ii) a radical of the formula

in which n is an integer from 2 to 12: and

(iii) the phenylene-Z-phenylene- radical in which Z represents —S—, —SO—, —SO_2—, —S—S—, —CH_2— or —NH—, and wherein the phenylene groups may be substituted by halogen atoms, methyl groups or methoxyl groups, the total number of carbon atoms in X and Y directly interposed between the two pairs of nitrogen atoms linked by X and Y respectively being at least 17,

which comprises reacting a diamine having the formula RHN—Y—NHR with a bisdicyandiamide having the formula



wherein R, X and Y have the meanings defined above,

or reacting a diamine having the formula RHN—Y—NHR or an inorganic salt thereof with a diamine salt of dicyanimide having the formula

at least the initial part of the reaction being carried out in the presence of a hydroxylic liquid which is a solvent for the said reactants, R, X and Y having the meanings defined above,

or reacting a mixture of a diamine having the formula RHN—Y—NHR and a monoamine of the formula R^1R^2NH with the product obtained by reacting 1 mol proportion of dicyanimide or an equivalent amount of a metal salt thereof with approximately 0.5 mol proportion of a diamine of the formula RHN—X—NHR, wherein R, X and Y have the meanings defined above, R^1 represents a hydrogen atom or a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms and R^2 represents a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms.

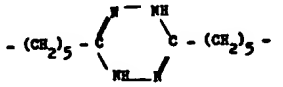
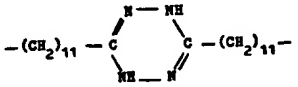
The preparation of polymeric biguanides by reaction of a diamine with a bisdicyanodiamide is fully described in U.K. Specification No. 702,268 already referred to.

The preparation of polymeric biguanides by reaction of a diamine or an inorganic salt thereof with a diamine salt of dicyanimide, at least the initial part of the reaction being carried out in the presence of a hydroxylic liquid which is a solvent for the said reactants, is fully described in U.K. Specification No. 1,152,243.

The preparation of polymeric biguanides by reaction of a mixture of a diamine and a monoamine with the product obtained by reacting 1 mol proportion of dicyanimide or an equivalent amount of a metal salt thereof with approximately 0.5 mol proportion of the same or a different diamine is fully described in U.K. Specification No. 1,167,249.

The radical Y may comprise saturated or unsaturated, homocyclic or heterocyclic nuclei, in which the number of carbon atoms directly interposed between the nitrogen atoms linked by Y is taken as including that segment of the cyclic group or groups which is the shortest.

Examples of polymeric biguanides within the scope of the present invention are given below, and are defined by the divalent radicals X and Y, X being hexamethylene in each case:—

Polymer No.	Y
1.	—$(CH_2)_6NHCO(CH_2)_4CONH(CH_2)_6$—
2.	
3.	
4.	
5.	—$(CH_2)_6$—NHCOCONH—$(CH_2)_6$—

By the methods of preparation indicated above, the polymeric biguanides are generally prepared in aqueous medium and the diamine RHN—Y—NHR is employed in the form of an inorganic salt thereof, usually the hydrochloride. Consequently, the polymeric biguanides are themselves usually obtained as their hydrochloride salts, which tend to have a considerable degree of water-solubility. Other salts of the polymeric biguanides are readily obtained either by varying the inorganic salt of the diamine RHN-Y—NHR from which the biguanide is made or by isolating the free base form of the biguanide from its hydrochloride or other salt and neutralising the free base with any desired acid. The latter method is preferred. In this way a large number of salts containing different anions may be obtained; and since different salts have different solubility characteristics, a range of biguanides from very sparingly soluble in water to highly soluble is readily available. By the choice of suitable anions, solubility in non-aqueous solvents can be achieved. Mixed salts may be prepared. Examples of polymeric biguanide salts are the sulphate, phosphate, acetate, tartrate, citrate, stearate, behenate, adipate, benzoate and phthalate.

The polymeric biguanides obtained by a process according to the present invention find application as bactericides and fungicides in a wide variety of different environments, and are particularly useful in agricultural situations. Thus, growing crops, plants, seeds, soil or harvested produce may be treated by any of the well-known and established procedures used in agriculture and crop protection. For example, the polymeric biguanides may be applied as solids, liquids, solutions, dispersions, emulsions and these may comprise, in addition to the active polymeric biguanide, any other adjuvant useful for formulation purposes, or any other biologically active substance, for example to increase the number of diseases combated.

Such solid or liquid substances and formulations may be applied, for example, by any conventional technique, for example by dusting, or otherwise applying the solid substances and formulations to the surfaces of growing crops, harvested produce, plants, seeds or soil, or to any part, or combination of parts thereof, or, for example, applying liquids or solutions for example, by dipping, spraying, mist blowing or soaking techniques.

The invention process is therefore useful for treating plants, seeds, harvested fruits, vegetables, or cut flowers infested with, or liable to infestation with fungal or bacterial diseases.

The term "seeds" is intended to include propagative plant forms generally and therefore includes, for example, cut stems, corms, tubers and rhizomes.

The polymeric biguanides, or salts thereof, may be used as such but are preferably formulated into compositions for this purpose.

In a further aspect, therefore, the invention provides a fungicidal or bactericidal composition for treating growing crops comprising, as an active ingredient, a polymeric biguanide as hereinbefore defined, together with a carrier substance therefor. The carrier may be a solid or liquid diluent. In the case of a liquid diluent being used, for example water, the composition may also contain a surface active (wetting) agent.

The compositions of the invention may be in the form of dusting powders or granules wherein the active ingredient is mixed with a solid diluent or carrier. Suitable diluents or carriers may be, for example kaolin, bentonite, kieselguhr, dolomite, calcium carbonate, talc, powdered magnesia, Fuller's earth, gypsum, Hewitt's earth, diatomaceous earth and China clay. Compositions for dressing seed, for example, may comprise in agent assisting the adhesion of the composition to the seed, for example a mineral oil.

The compositions may also be in the form of dispersible powders or grains comprising, in addition to the active ingredient, a wetting agent to facilitate the dispersion of the powder or grains in liquids. Such powders or grains may include fillers and suspending agents.

The compositions may also be in the form of liquid preparations to be used in the process of the invention for plants or harvested produce which are generally solutions, aqueous dispersions or emulsions containing the active ingredient in the presence of or one or more wetting agents, dispersing agents, emulsifying agents or suspending agents.

Wetting agents, dispersing agents and emulsifying agents may be of the cationic, anionic or non-ionic type. Suitable agents of the cationic type include, for example quaternary ammonium compounds, for example, cetyltrimethyl ammonium bromide. Suitable agents of the anionic type include for example, soaps, salts of aliphatic mono-

esters or sulphuric acid, for example sodium lauryl sulphate, salts of sulphonated aromatic compounds, for example sodium dodecylbenzenesulphonate, sodium, calcium or ammonium lignosulphonate butyl-naphthalene sulphonate, and a mixture of the sodium salts of diisopropyl and triisopropylnaphthalene sulphonic acids. Suitable agents of the non-ionic type include, for example, the condensation products of ethylene oxide with fatty alcohols for example oleyl alcohol or cetyl alcohol, or with alkyl phenols for example octylphenol, nonylphenol and octylcresol.

Other non-ionic agents are the partial esters derived from long chain fatty acids and hexitol anhydrides, the condensation products of the said partial esters with ethylene oxide, and the lecithins. Suitable suspending agents are, for example hydrophilic colloids, for example polyvinylpyrrolidone and sodium carboxymethylcellulose, and the vegetable gums for example gum acacia and gum tragacanth.

The non-aqueous solutions, dispersions or emulsions may be prepared by dissolving the active ingredient in an organic solvent which may contain one or more wetting, dispersing or emulsifying agents. Suitable organic solvents are ethylene dichloride, isopropyl alcohol, propylene glycol, diacetone alcohol, toluene, kerosene, methylnaphthalene, xylenes and trichloroethylene.

The compositions to be used as sprays may also be in the form of aerosols wherein the formulation held in a container under pressure in the presence of a propellant for example fluorotrichloromethane or dichlorodifluoromethane.

By the inclusion of suitable additives, for example for improving the distribution, adhesive power and resistance to rain on treated surfaces, the different compositions can be better adapted for the various uses for which they are intended.

The compositions may also be conveniently formulated by admixing them with fertilizers. A preferred composition of this type comprises granules of fertilizer material incorporating a polymeric biguanide obtained by the process of the present invention. The fertilizer material may, for example, comprise nitrogen or phosphate-containing substances.

The compositions which are to be used in the form of aqueous dispersions or emulsions are generally supplied in the form of a concentrate containing a high proportion of the active ingredient, the said concentrate to be diluted with water before use.

The concentrates are often required to withstand storage for prolonged periods and after such storage, to be capable of dilution with water in order to form aqueous preparations which remain homogeneous for a sufficient time to enable them to be applied by conventional spray equipment.

The concentrates may conveniently contain from 4—85% and generally from 4—60% by weight of the active ingredient. A 20% aqueous solution is preferred. When diluted to form aqueous preparations, such preparations may contain varying amounts of the active ingredient depending upon the purpose of which they are to be used, but an aqueous preparation containing between 0.001% and 10% by weight of active ingredient may generally be used for the control of micro-organisms by contacting the latter with the preparation.

It is understood that the compositions of this invention may comprise, in addition to one or more polymeric substances according to the invention, one or more other substances having biological activity, for example fungicidal, bactericidal, or insecticidal activity.

The invention is illustrated but not limited by the following Examples in which parts and percentages are by weight unless otherwise stated, and in which the ratio of parts by weight to parts by volume is that of the kilogram to the litre.

Polymer numbers mentioned in the Examples correspond with the numbers allocated to individual polymers in the preceding table, and are included for ease of identification.

Example 1.

Preparation of a polymeric biguanide having
$X=-(CH_2)_6-$ and $Y=-(CH_2)_6NHCOCONH(CH_2)_6-$
(Polymer No. 5)

A mixture of N,N′ - bis(6 - aminohexyl)oxamide (14.3 parts), concentrated hydrochloric acid (8.73 parts by volume), water (53 parts) and a solution of hexamethylenediamine bis(dicyanimide) salt (12.5 parts) in water (29.2 parts) is stirred and heated to remove the water. The temperature of the residue is raised to 140°C and maintained at this level for 2 hours. The product is cooled to about 100°C and water (120 parts) is added. After stirring the mixture at 90°C for 13 minutes a clear

solution is obtained which on cooling yields a white paste (148.9 parts) having a solids content of 22.5%.

The U.V. spectrum of the paste showed a peak at 232 mm characteristic of a biguanide compound.

In an antibacterial test against *Escherichia coli* using the standard test method described below, 200 parts per million of the above product (calculated at 100% strength) reduced the bacterial count from $>3\times10^7$ to <10.

Standard Test Method for the Determination of Bactericidal Activity

A suspension of *Escherichia coli* is prepared from a 24 hour glucose nutrient agar culture. 1 ml of this suspension is added to 9 ml of biocide solution to provide 10 ml of suspension containing the required level of biocide.

After a contact period of 3 minutes at 22°C 1 ml of the test mixture is transferred into 9 ml of 0.5% Antrypol solution (in order to neutralise the bactericidal activity of the biocide). The Antrypol solution provides the first decimal dilution and 1 ml of this solution is then further decimally diluted in a series of 9 ml volumes of saline solution (0.8%) by means of which the surviving cells are determined in nutrient agar.

Example 2.

Preparation of a polymeric biguanide having

$X=-(CH_2)_6-$ and $Y=-(CH_2)_6NHCO(CH_2)_4CONH(CH_2)_6-$

(Polymer No. 1)

To a stirred mixture of N,N' - bis(6 - aminohexyl)adipamide (10.3 parts), hexamethylene diamine bis(dicyanimide) salt (7.5 parts) and water (37.5 parts) is added 22 parts by volume of a hydrochloric acid solution prepared by adding water to concentrated hydrochloric acid (6 parts by volume) to give 25 parts by volume of solution. The mixture is heated at 75—80°C for 35 minutes to give a turbid solution having a pH of 4 to 5. The temperature of the solution is then raised to 145°C during which period the water is removed by distillation. After 2 hours at 140—145°C the temperature is reduced to 120°C and 72 parts of water are added over a period of 20 minutes.

The mixture is then boiled under reflux for 35 minutes, cooled, filtered to remove some solid material (2 parts, rejected), and yields a clear solution containing 21% by weight of the polymeric biguanide, identified by its characteristic U.V. spectrum (peak at 232mm).

In an antibacterial test, conducted as described in Example 1, against *E.coli* using 200 p.p.m. of the above produce (calculated at 100% strength), the bacterial count was reduced from $>3\times10^7$ to <10.

Example 3.

Preparation of a polymeric biguanide having

$X=-(CH_2)_6-$ and $Y=$ 

(Polymer No. 3)

3,6 - Bis - (5' - aminopentyl) - 1,4 - dihydro - 1,2,4,5 - tetrazine trihydrochloride (12.12 parts) and 1,6 - bis - (cyanoguanidino)hexane (8.33 parts) are mixed with water (100 parts) and the mixture is heated at 100°C for 1 hour. The water is removed by distillation and the residue is heated at 145°C for 2 hours, after which time it is dissolved in hot water to give a 20% solution. This material gave a UV spectrum (peak at 232 mm) characteristic of biguanides.

In an antibacterial test, conducted as described in Example 1, against *E.coli* using 200 p.p.m. of the above product (calculated at 100% strength), the bacterial count was reduced from $>3\times10^7$ to <10.

3,6 - Bis - (5' - aminopentyl) - 1,4 - dihydro - 1,2,4,5 - tetrazine trihydrochloride is obtained by the following method:—

A mixture of caprolactam (22.6 parts) and hydrazine dihydrochloride (21.0 parts) is stirred and heated in a stream of nitrogen at 180°—210°C for six hours. Hydrogen chloride is evolved. After cooling, the product is dissolved in water (200 parts) and the solution is extracted with chloroform (2×50 parts) to remove any unreacted lactam. The water is removed from the solution under vacuum leaving the

tetrazine as an off-white solid (45.5 parts) (see U.S. Patent Specification No. 3,629,249).

Example 4.

Preparation of a polymeric biguanide having

$X = -(CH_2)_6-$ and Y = 

(Polymer No. 4)

This preparation is carried out exactly as described in Example 3 except that the 12.12 parts of 3,6 - bis - (5′ - aminopentyl) - 1,4 - dihydro - 1,2,4,5 - tetrazine trihydrochloride are replaced by 14.38 parts of 3,6 - bis - (11′ - aminoundecyl)- 1,4 - dihydro - 1,2,4,5 - tetrazine trihydrochloride. The product obtained has similar activity against *E.coli*.

3,6 - Bis - (11′ - aminoundecyl) - 1,4 - dihydro - 1,2,4,5 - tetrazine trihydrochloride is obtained by a similar method to that described for the preparation of the corresponding 5′-aminopentyl derivative in Example 3, the 22.6 parts of caprolactam being replaced by 39.4 parts of laurolactam.

Other polymeric biguanides which are disclosed herein or which come within the scope of the present invention are obtained by similar methods of preparation to those described in the foregoing Examples, using diamines appropriately selected to provide the desired structure in the resulting product.

Example 5.

Certain polymeric biguanides of the invention were tested against a variety of foliar fungal diseases of plants. The technique employed is to spray the foliage of the undiseased plants with a solution of the test polymer and also to drench the soil in which the plants are growing with another solution of the test polymer.

All solutions for spraying contained 0.02% of the test polymer. All the soil drench solutions also contained 0.02% of the test polymer.

The plants were then infected with the disease it was desired to control and after a period of days, depending upon the particular disease, the extent of the disease was visually assessed. The results are given below, in the form of a grading as follows:—

Grading	Percentage Amount of Disease
0	61 to 100
1	26 to 60
2	6 to 25
3	0 to 5

In the first Table below, the disease is given in the first column, whilst in the second column is given the time which elapsed between infecting the plants and assessing the amount of disease. The third column assigns to each disease a code letter, these code letters being used in the Second Table to identify the diseases.

TABLE

Disease and Plant	Time Interval (days)	Disease Code Letter (Table No. 2)
Plasmopara viticola (vine)	7	A
Botrytis cinerea (broad bean)	3	B

TABLE (Continued)

Polymer No. and Rate of Application	Disease Code Letter	
	A	B
1	0	3
2	0	3
3	2	3

In further biological tests which were conducted certain polymeric biguanides of the invention (Nos. 2 and 3) displayed *in vitro* anti-bacterial activity against a number of bacterial plant pathogens. These two polymeric biguanides displayed growth-stunting effects upon the plants used in the *in vitro* anti-bacterial tests. One polymer, No. 1 displayed plant anti-viral activity against the disease potato virus Y.

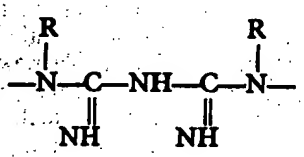
In tests conducted against algae the polymeric biguanides numbered 1, 2 and 3 all displayed a high level of activity against algae.

WHAT WE CLAIM IS:—

A process for the preparation of polymeric biguanides and salts thereof in which the recurring polymer unit has the general formula:

—X—BG—Y—BG—

wherein BG represents the biguanide radical



in which

each R independently represents a hydrogen atom or a hydrocarbon radical;

X represents a polymethylene radical having from 2 to 12 carbon atoms or a radical the same as Y;

Y represents a divalent radical selected from

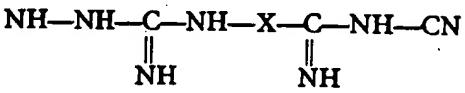
(i) symmetrical aliphatic, aromatic or aliphatic-aromatic radicals having one or two amide or urea linkages therein;

(ii) a radical of the formula

in which n is an integer from 2 to 12; and

(iii) the phenylene-Z-phenylene- radical in which Z represents —S—, —SO—, —SO_2—, —S—S—, —CH_2 or —NH, and wherein the phenylene groups may be substituted by halogen atoms, methyl groups or methoxyl groups, the total number of carbon atoms in X and Y directly interposed between the two pairs of nitrogen atoms linked by X and Y respectively being at least 17,

which comprises reacting a diamine having the formula RHN—Y—NHR with a bisdicyandiamide having the formula



wherein R, X and Y have the meanings defined above,

or reacting a diamine having the formula RHN—Y—NHR or an inorganic salt of dicyanimide having the formula

$$[RH_2N—X—NH_2R]^{+}\,[N(CN)_2]_2^{-},$$

at least the initial part of the reaction being carried out in the presence of a hydroxylic liquid which is a solvent for the said reactants, R, X and Y having the meanings defined above,

or reacting a mixture of a diamine having the formula RHN—Y—NHR and a monoamine of the formula R^1R^2NH with the product obtained by reacting 1 mol proportion of dicyanimide or an equivalent amount of a metal salt thereof with approximately 0.5 mol proportion of a diamine of the formula RHN—X—NHR, wherein R, X and Y have the meanings defined above, R^1 represents a hydrogen atom or a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms and R^2 represents a substituted or unsubstituted aliphatic, cycloaliphatic, araliphatic or aromatic hydrocarbon radical containing from 1 to 18 carbon atoms.

2. A process for the preparation of polymeric biguanides substantially as hereinbefore described in any one of the foregoing Examples 1 to 4.

3. Polymeric biguanides and salts thereof whenever obtained by a process as claimed in claim 1 or claim 2.

4. A fungicidal or bactericidal composition comprising, as an active ingredient, a polymeric biguanide as claimed in claim 3, together with a carrier substance therefor.

5. A composition as claimed in claim 4 wherein the active ingredient is mixed with a solid diluent or carrier.

6. A composition as claimed in claim 5 wherein the solid diluent or carrier is kaolin, bentonite, kieselguhr, dolomite, calcium carbonate, talc, powdered magnesia, Fuller's earth, gypsum, Hewitt's earth, diatomaceous earth or China clay.

7. A seed-dressing composition which comprises a composition as claimed in claim 5 or claim 6 and an agent assisting the adhesion of the composition to the seed.

8. A seed-dressing composition as claimed in claim 7 wherein the agent assisting the adhesion of the composition to the seed in a mineral oil.

9. A composition in the form of a dispersible powder or grains which comprises a composition as claimed in claim 5 or claim 6, and a wetting agent.

10. A composition as claimed in claim 4 which comprises an aqueous or non-aqueous solution, dispersion or emulsion containing the active ingredient in the presence of one or more wetting agents, dispersing agents, emulsifying agents or suspending agents.

11. An aerosol which comprises a polymeric biguanide or salt thereof claimed in claim 3 held in a container under pressure in the presence of a propellant.

12. An aerosol as claimed in claim 11 wherein the propellant is fluorotrichloromethane or dichlorodifluoromethane.

13. A fertiliser composition, comprising granules of fertiliser material incorporating a polymeric biguanide or salt thereof claimed in Claim 3.

14. An aqueous concentrate of a polymeric biguanide claimed in claim 3 containing from 4 to 85% by weight of the active ingredient.

15. An aqueous concentrate as claimed in claim 14 which contains from 4 to 60% by weight of the active ingredient.

16. A 20% by weight aqueous solution of a polymeric biguanide or salt thereof claimed in claim 3.

17. A method for the control of micro-organisms which comprises contacting the micro-organisms with an aqueous preparation of a polymeric biguanide or salt thereof claimed in claim 3, the preparation containing between 0.001% and 10% by weight of active ingredient.

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